REMARKS

The present response is being made during the first month following the three months statutorily provided for response. Enclosed please find a request to purchase an additional month for response from July 27, 1998 to August 27, 1998 along with the necessary fee.

In the Office Action the Examiner objected to the Specification because an abstract was not provided on a separate sheet as required by 37 CFR 1.72(b). The above-amendment enters the Abstract which was apparently omitted from the copy sent to the Patent Office. Applicants acknowledge the problem with use of the trademark TRITON® which oversight has been corrected in the above-amendments.

Because the Examiner did not renew the original rejections under 35 U.S.C. § 102, 35 U.S.C. § 103, and 35 U.S.C. §112, Applicants presumes that the previously made amendments resulted in the withdrawal of these rejections.

Applicants hereby confirms the Examiner's presumption that the various claims of the instant application were all made by both named inventors and were commonly owned at that time.

In the Final Office Action the Examiner issued a NEW GROUND of rejection of Claims 1-20. The Examiner found that these claims were unpatentable for obviousness (35 U.S.C. § 103) over **Gorczyca et al.** (Cancer Research 53:1945-52 (1993)), in view of **Vanderlaan et al.** (U.S. Patent No 5,053,336), and **X. Li et al.** (Int. J. Oncology 1157-1161 (1994)).The Examiner

reasoned that **Gorczyca et al.** teaches the use of TdT to label the ends of nuclear DNA strand breaks with biotinylated nucleotides. The biotinylated label is subsequently detected *in situ* with a labeled antibody. However, **Gorczyca et al.** does not teach the use of halogenated nucleotides to label DNA strand ends. **Vanderlaan et al.** teaches the detection of halogenated nucleotides added to double stranded DNA during normal DNA synthesis. The halogenated nucleotides are subsequently detected by antibodies after a denaturation step. **Vanderlaan et al.** does not teach the use of halogenated nucleotides to label strand breaks. Finally, **X. Li et al.** teaches the use of halogenated nucleotides incorporated during normal DNA synthesis (as in **Vanderlaan et al.**) to induce strand breaks by photolysis. These newly-created breaks are subsequently labeled by with biotinylated nucleotides (as in **Gorczyca et al.**). This method does not require a denaturation step. The Examiner then concludes that one of ordinary skill in the art would combine the teaching of **Vanderlaan et al.** (labeling double strands with halogenated nucleotides) with **Gorczyca et al.** (TdT labeling of broken strands with biotinylated nucleotides) and with **X. Li et al.** (labeling of broken strands with biotinylated nucleotide does not require denaturation) to yield the instant invention.

Claim Rejections Under 35 U.S.C. § 103

Applicants respectfully traverse this finding of obviousness and request consideration of the following remarks. The instant invention is the discovery of the greatly superior results of using halogenated nucleotides to **end label** DNA. As explained in the Specification (see page 15, Table 1 and related text), not only is halogenated nucleotide economically superior to biotinylated or digoxygenin methods, the halogenated nuceotide method of the instant invention

is unexpectedly almost twice as good as the next best method (digoxygenin). Prior to the instant invention **no one** had shown that halogenated precursors could be used to label DNA strand ends. The Examiner attempts to reduce the significance of this finding by reasoning that "Vanderlaan taught that a halogenated nucleotide binds to the 3' end of DNA strands during extension in normal DNA synthesis." Applicants respectfully remind the Examiner that this is not a fair summary of the teachings of **Vanderlaan et al.** That reference teaches the enzymatic addition of halogenated nucleotides during normal DNA synthesis. Normal DNA synthesis is **not** "extension." Further, during normal semi-conservative DNA replication there are **no strand ends** in the sense used in the present invention where both the 5' and the 3' polymer chains are broken leaving a double helix strand end. There is, instead, a 3' chain end where the newly synthesized chain grows as complementary bases bind to the intact 5' chain and are added to the 3' chain. Prior to the instant invention no one had shown that enzymes capable of strand end labeling (extension by adding to one of the chains, 3' in the case of TdT) would add halogenated nucleotides to a strand end.

Considering that halogen groups (bromine in this case) are fairly bulky one might easily expect that there might be some type of steric hindrance as the enzyme attempted to add halogenated nucleotides to the chain. Considering that halogenated nucleotides are considerably less expensive than either biotinylated or digoxygenin nucleotides there should have been a strong economic motivation for others to make the instant discovery if the Examiner's obviousness finding is valid. However, the instant invention was not made because labeling of double stranded DNA with halogenated precursors during normal DNA synthesis simply is not

the same and does not readily suggest using halogenated precursors to end label DNA strands. Once the instant inventors discovered that such labeling was possible, the other unexpected advantages of the instant invention became apparent. As the Examiner has pointed out, biotin end labeled DNA can bind specific antibodies without denaturation. In fact, biotin labeled double stranded DNA can also bind antibodies without denaturation because the position of the biotin on the DNA polymer does not bury the biotin within the helix. On the other hand, halogenated labels are located within the ring of the nucleotide base and are buried within the double helix, thus requiring denaturation—opening up of the helix—for an antibody to bind to the halogen. Lack of denaturation with biotinylated labels has no necessary predictive value towards denaturation (or lack there of) with halogen labels. If one could get an enzyme to add halogenated nucleotides to just one of the chains of a helix, the halogen might be out in the open for antibody binding without denaturation. However, the key step is still demonstrating that such labeling with halogenated nucleotides can occur. The cited prior art does not teach or render obvious this step.

Finally, as demonstrated in the specification the method of the present invention produces superior signal to noise ratios to other methods available for labeling DNA strand ends. This is a totally unexpected discovery. Not only is the instant invention simple and comparatively inexpensive, it produces significantly superior results to competing methods. There is nothing in the prior art to teach or suggest that such superiority would be attained with halogenated precursors as opposed to digoxygenin or other labels. Applicants were the first to discover this

significant advantage and deserve patent protection for this discovery. Similarly, Applicants were the first to discover that halogenated nucleotides could be effectively added to DNA helix ends.

Applicant respectfully submits that the case is now in condition for allowance and requests an early notification of the same. Questions, suggestions, and comments from the Examiner are welcomed. If the Examiner believes that a telephone conference would help further the prosecution of the case, the Examiner is requested to contact the undersigned attorney at the listed telephone number.

Respectfully submitted,

GRAHAM & JAMES LLP

Stefan J. Kirchanski
Registration No. 36, 568
650 Town Center Drive, Sixth Floor
Costa Mesa, California 92626
Telephone: 714/751-8800